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Attraction of ambrosia beetles to ethanol baited traps in a Slovakian oak forest

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Abstract: The attractiveness of ultra high release ethanol lures to ambrosia beetles in Slovakian oak forests was tested from 2010 to 2012. A total of 24,705 specimens were captured during this three year period with *Xyleborinus saxesenii* (Ratzeburg, 1837) representing 49.28% (12,174 specimens) of the total. Other dominant species captured in the traps were *Anisandrus dispar* (F., 1792) (27.84%), *Xyleborus monographus* (F., 1792) (9.72%) and *Trypodendron signatum* (F., 1792) (6.04%). During this experiment, *Xylosandrus germanus* (Blandford, 1894) was detected for the first time in Slovakia with an increase in capture each year (19, 40 and 77 specimens, respectively). Flight period for ambrosia beetles in Slovakia occurs from the beginning of April through the end of September. This is the first time that ethanol baited traps were deployed in Slovakian oak forests and the lures were an effective tool for monitoring native and non-native ambrosia beetles.

Key words: ambrosia beetles; ethanol; oak; *Quercus*; trap; lures

Introduction

Oak forests in Europe have been in decline for many years (Leontovyč & Čapek 1987; Führer 1998; Sonesson & Drobyshev, 2010). Weakened oaks are often attractive to many bark and wood boring insects including ambrosia beetles.

Ambrosia beetles belong to the subfamilies Scolytinae and Platypodinae (Coleoptera: Curculionidae) (Knížek & Beaver 2004). Many species are polyphagous and breed in deciduous broadleaf trees, shrubs and coniferous species.

Many ambrosia beetles use ethanol as a primary attractant in locating suitable host material (Ranger et al. 2010). Several studies suggest that ethanol is produced from fermentative processes in trees in response to various environmental stresses including flooding, drought, or high levels of pollutant gases (Kimmerer & Kozlowski 1982; Montgomery & Wargo 1983; Naik et al. 2010). The attraction of ambrosia beetles to ethanol is related to their preference for woody material that has sufficiently aged to allow anaerobic respiration to generate ethanol within the tissues (Graham 1968; Cade et al. 1970; Moeck 1970; Lindelöw et al. 1993; Miller & Rabaglia 2009). The detection of ethanol indicates the presence of suitable habitats for numerous insects (Klimetzek et al. 1986).

The use of ethanol baited traps for monitoring of native and non-native ambrosia beetles is not widely used in Europe. There are only a few published studies where ethanol lures have been used: oak forests in Greece (Markalas & Kalapanida 1997) and France (Bouget & Noblecourt 2005). There are no published studies where ethanol has been used to monitor ambrosia beetles in Slovakia or adjacent countries.

In contrast, ethanol baited traps are commonly used to assess temporal and spatial patterns of ambrosia beetles, for monitoring ambrosia beetles in ornamental nurseries, and they are used in various detection programs in the USA and Canada (Humble 2001; Sweeney et al. 2007; Miller & Rabaglia 2009; Reding et al. 2010; Ranger et al. 2011; Noseworthy et al. 2012; Bullas-Appleton et al. 2014).

The aim of our study was (i) to test the attractiveness of ethanol baited traps to ambrosia beetles in a selected oak forest in W Slovakia; (ii) to evaluate the diversity of ambrosia beetles in this forest; (iii) to assess ambrosia beetle flight patterns in W Slovakia; (iv) to determine the elution rate of ethanol from commercially available lures; and (v) to confirm the presence of *Xylosandrus germanus* (Blandford, 1894) in Slovakia.

Material and methods

Experimental site

Research was conducted between 2010–2012 in the Prievidza Forest District near Duchonka (48°40′ N, 18°05′ E) in

Table 1. Description of lure treatments over 3 years.

Year	Replicates	Lure type	Nr. of traps	Trapping dates
2010	8	UHR ethanol, control	16	26 March–10 September 2010 (lures changed 18 June)
2011	5	UHR ethanol, UHR ethanol + 2-methyl-3-buten-2-ol, UHR ethanol + (E)-2-hexenal, UHR ethanol + (E)-conophthorin, control	25	24 March–9 September 2011 (lures changed 17 June)
2012	6	UHR ethanol, control	12	23 March–7 September 2012 (lures changed 15 June)

W Slovakia [Köppen climate classification (KCC) *Cfb*] (Kottek et al. 2006). The average elevation of the site is approximately 300 m a.s.l. The forest is dominated by 60 to 80 year old oak (*Quercus* spp.). The predominant tree species are *Q. dalechampii*, *Q. robur*, *Q. petrae* and *Q. cerris*.

Traps and lures

Lindgren funnel traps (12 unit, wet option) (Contech Enterprises Inc., Victoria, BC, Canada) (Lindgren 1983) baited with ultra high release gelled ethanol (Contech Enterprises Inc., Victoria, BC, Canada), (hereinafter referred to as "UHR ethanol") were used. Each lure contains approximately 125 g of 95% ethanol and has a release rate of 260 mg/day at 20 °C (Contech Enterprises Inc., pers. comm.). Traps were installed on 2.0–2.2 m tall wooden stands and spaced 15–20 m apart. Approximately 100 ml of propylene glycol ($C_3H_8O_2$, 99.5%) (Penta-Ing. Petr Švec, Prague, Czech Republic), diluted with water (1:1) and some soap to break the water tension, was placed into the collection jar at the bottom of the trap. This mixture served to preserve captured specimens and thus facilitate identification. Traps were installed in the field and baited with ethanol lures at the end of March and lures were replaced in the middle of June.

Every two weeks, samples were collected from each trap. Each sample was placed in separate containers for transportation back to the laboratory. The samples were stored in the refrigerator at the 4 °C. Ambrosia beetle species were determined according to the insect identification key presented by Pfeffer (1989). Samples of trapped species are archived at the National Forest Centre, Forest Research Institute Zvolen, Forest Protection Service (Banská Štiavnica, Slovakia).

In 2010, 8 traps baited with one UHR ethanol lure and 8 control traps (no lure) were placed in the field. In 2012 the number of traps differed from that in 2010, with 6 traps baited with one UHR ethanol lure and 6 control traps (no lure). In 2011, 3 other lure types were tested. In addition to 5 traps baited with UHR ethanol and 5 control traps, there were 5 traps baited with UHR ethanol + 2-methyl-3-buten-2-ol, 5 traps baited with UHR ethanol + (*E*)-2-hexenal and 5 traps with UHR ethanol + (*E*)-conophthorin (Contech Enterprises Inc., Victoria, BC, Canada) (Table 1).

Elution of ethanol from the release devices

At the beginning and the end of 2011 and 2012 seasons, the UHR ethanol lures were weighed to determine actual release rates. Boeco BBL 31 (precision: 0.0001 g, capacity: 210 g) (Boeco Germany, Hamburg, Germany) balances were used. The lures were weighed to the nearest 0.01 g. We did not weigh the other lures nor the UHR ethanol lures from 2010.

Statistical analysis

The results of trap catches were statistically evaluated using one- and two-factor analysis of variance (ANOVA). To help stabilize the variance, trap catch data were $\log(x+1)$ transformed but untransformed data are presented. We used the HSD (Honestly Significant Difference) Tukey test to determine the minimum difference between means. In all cases, $\alpha = 0.05$ was used to determine statistical significance. All statistical analyses were performed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA).

Results

Trapping of ambrosia beetles

A total of 24,705 ambrosia beetles of 11 different species were collected in baited (UHR ethanol; UHR ethanol + other chemicals) and unbaited (control) traps during the three year period (Table 2). A total of 12,174 *Xyleborinus saxesenii* (Ratzeburg, 1837) specimens were trapped which represents 49.28% of all beetles captured. *X. saxesenii* specimens represented 60.68% (2010), 51.94% (2011) and 19.32% (2012) of the total trap catches. *Anisandrus dispar* (F., 1792) was the second most abundant beetle trapped with 6,878 specimens caught over three years (27.84%). The pattern of trapped *A. dispar* during the study period was relatively steady (26.29, 29.59 and 23.17%, respectively). X*yleborus monographus* (F., 1792) (9.72%) was trapped at relatively high proportions over the three year period. However, *Trypodendron signatum* (F., 1792) (6.04%) was only captured at high levels in 2012 (40.26%) (Table 2). *Platypus cylindrus* (F., 1792), *Xyleborinus attenuatus* (Blandford, 1894), *Xyleborus dryographus* (Ratzeburg, 1837), *X. germanus, Xyleborus pfeilii* (Ratzeburg, 1837), *Trypodendron domesticum* (L., 1758) and *Trypodendron lineatum* (Olivier, 1795) were also trapped, but each represented less than 5% of the total captures. *X. germanus* is a non-native species captured for the first time in Slovakia. The number of *X. germanus* specimens gradually increased over time from 19 (2010), 49 (2011) to 77 (2012) for a total of 136 over 3 years.

In 2010, the average number of *X. saxesenii* captured was 37.91 beetles/trap per catching interval (14 days) (Fig. 1A). In the same year, the average number of *A. dispar* caught was also quite high (16.42

Table 2. Total catches (N) and dominance (D%) of all ambrosia beetles caught during the study period.

Species	2010		2011		2012		2010–2012	
	N	D%	N	D%	N	D%	N	D%
Platypodidae								
Platypus cylindrus (F., 1792)	–	–	8	0.05	10	0.28	18	0.07
Curculionidae, Scolytinae								
Xyleborinus attenuatus (Blandford, 1894)	22	0.36	389	2.58	14	0.39	425	1.72
Anisandrus dispar (F., 1792)	1,582	26.29	4,455	29.59	841	23.17	6,878	27.84
Xyleborus dryographus (Ratzeburg, 1837)	372	6.18	696	4.62	84	2.31	1,152	4.66
Xylosandrus germanus (Blandford, 1894)	19	0.31	40	0.27	77	2.12	136	0.55
Xyleborus monographus (F., 1792)	347	5.77	1,616	10.73	439	12.10	2,402	9.72
Xyleborus pfeilii (Ratzeburg, 1837)	3	0.05	–	–	–	–	3	0.01
Xyleborinus saxesenii (Ratzeburg, 1837)	3,652	60.68	7,821	51.94	701	19.32	12,174	49.28
Trypodendron domesticum (L., 1758)	5	0.08	15	0.10	2	0.06	22	0.09
Trypodendron lineatum (Olivier, 1795)	–	–	2	0.01	–	–	2	0.01
Trypodendron signatum (F., 1792)	16	0.27	16	0.10	1,461	40.26	1,493	6.04
Total	6,018		15,058		3,629		24,705	
N species	9		10		9		11	

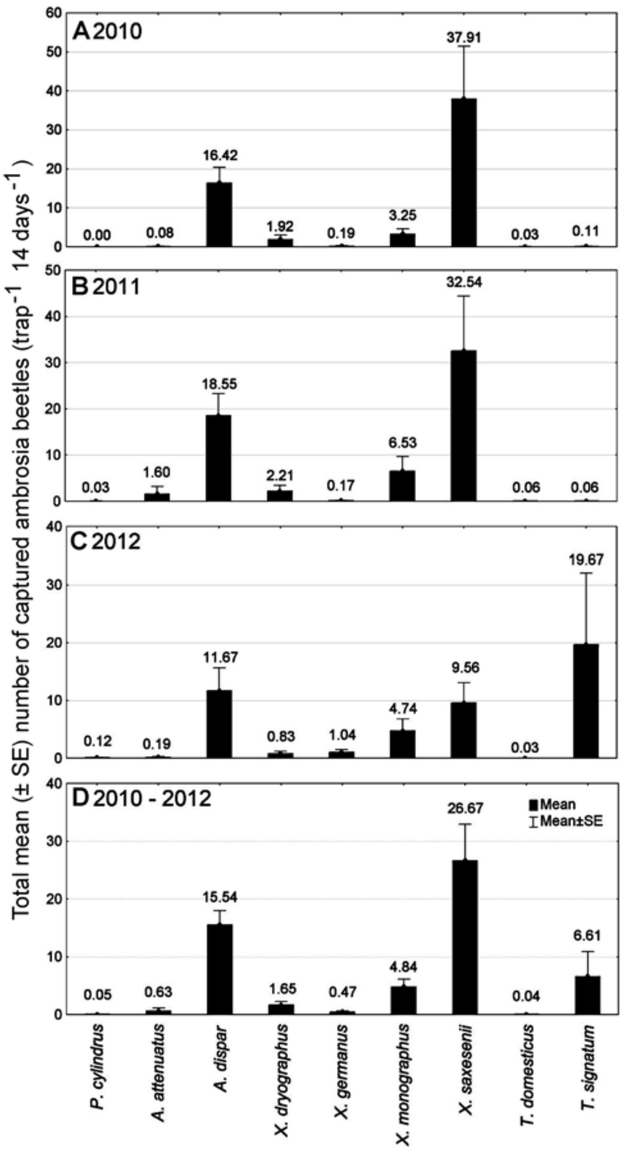

Fig. 1. Comparison of average captures of ambrosia beetles in each year (A–C) and for the entire experiment (D).

beetles/trap). The average number of all other ambrosia beetles trapped was less than 5 specimens/trap. The species captured most frequently in 2011 were also *X. saxesenii* (32.54 beetles/trap) and *A. dispar* (18.55 beetles/trap), followed by *X. monographus* (6.53 beetles/trap) (Fig. 1B). Surprisingly in 2012, the highest number of beetles caught were *T. signatum* (19.67 beetles/trap), followed by *A. dispar* (11.67 beetles/trap) and *X. saxesenii* (9.56 beetles/trap) (Fig. 1C). Over the entire trapping period, there was an average of 26.67 *X. saxesenii*, 15.54 *A. dispar*, 6.61 *T. signatum* and 4.84 *X. monographus* captured per trap. The average number of other species was less than 2 specimens/trap. *X. pfeilii*, *T. domesticum* and *T. lineatum* were not evaluated due to very low captures.

Seasonal flight patterns of nine ambrosia beetles are shown in Fig. 2. The peak of the most common ambrosia beetle, *X. saxesenii,* was late April/early May. Two large peaks were observed in 2010 (Fig. 2G). Flight activity of *A. dispar* was similar in 2010 and 2012 with a peak in May and another one in July. In 2011, peak flight activity occurred at the end of April and then it gradually declined (Fig. 2C). The highest catches of *X. attenuatus* occurred in 2011, with peak captures in early April. Low numbers were recorded in the other two years, however, flight still occurred in September in 2012 (Fig. 2B). Each year, the highest catches of *X. dryographus* were recorded in June and July, but in 2012 the quantity of captured beetles was much lower than in previous years (Fig. 2D). The peak activity of *X. germanus* occurred in mid-June and the number of beetles captured increased in 2012 (Fig. 2E). In 2010 and 2012, the seasonal flight pattern of *X. monographus* had one peak in May and another in early/mid-June, whereas in 2011, there was only one peak and it occurred towards the end of May (Fig. 2F). Except for *A. dispar* and *X. attenuatus*, *T. domesticum* emerges earlier in the year than the other ambrosia beetles. With the exception of 2012, peak flight of *T. domesticum* oc-

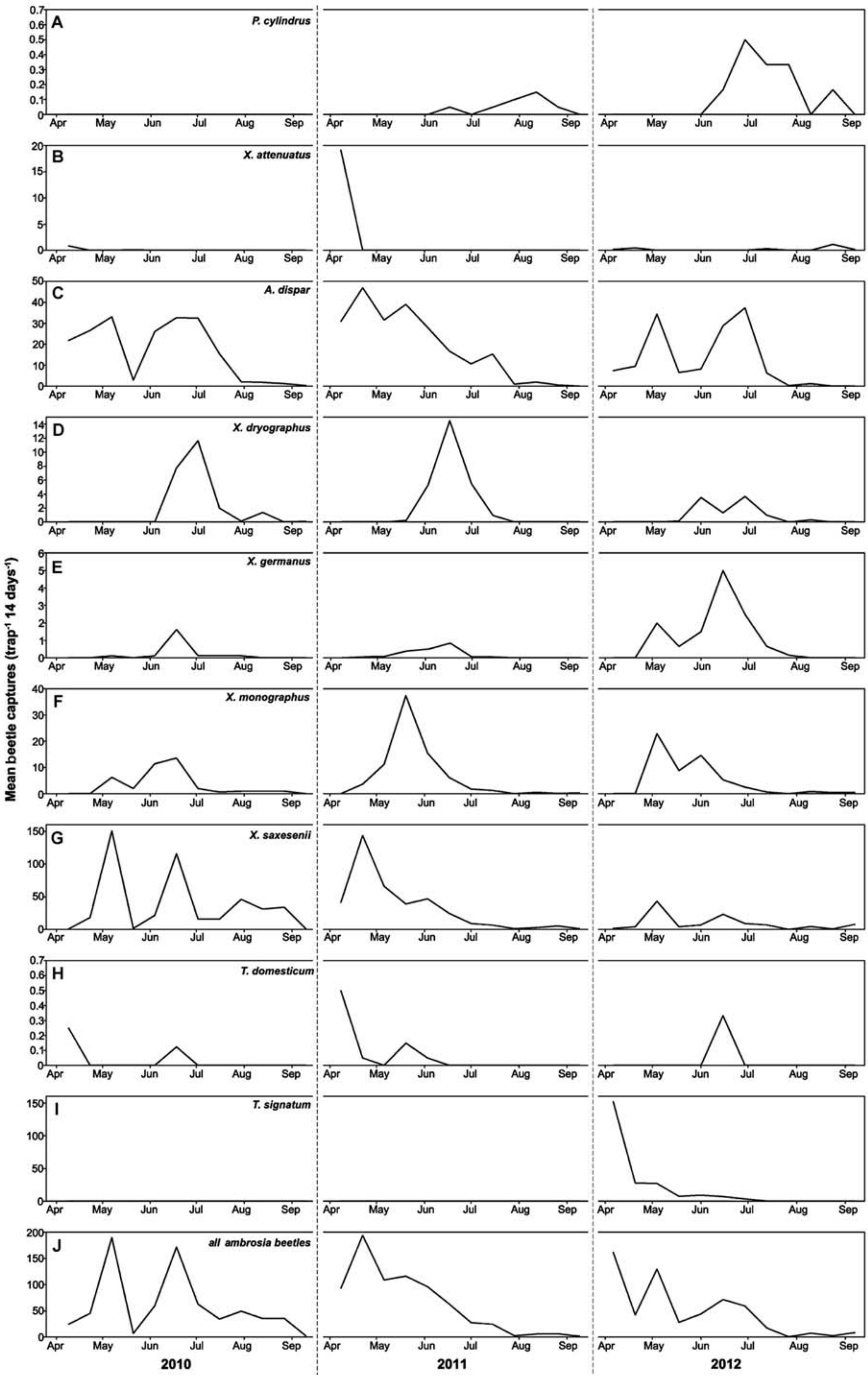

Fig. 2. Seasonal flight patterns of individual species (A–I) and of all ambrosia beetle species (J) captured in the period 2010–2012.

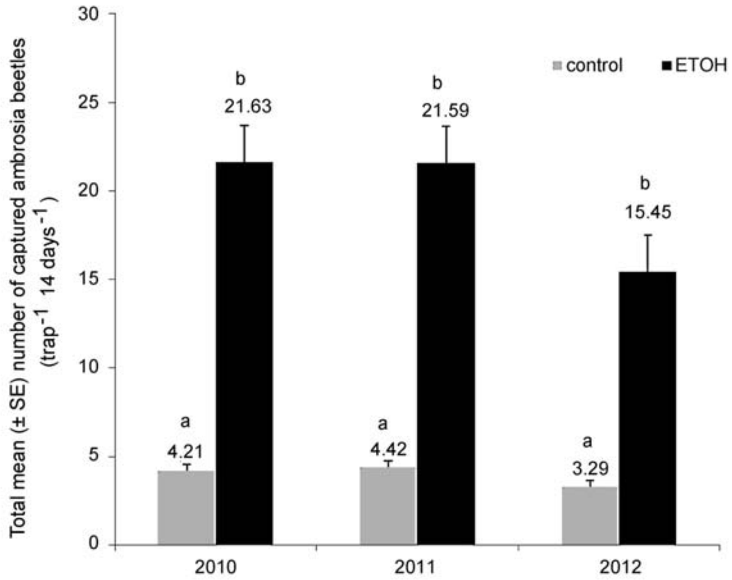

Fig. 3. Comparison of the total number of ambrosia beetles captured in control and UHR ethanol baited traps. Means followed by the same letter are not significantly different ($\alpha = 0.05$, HSD Tukey test).

Table 3. Elution of ethanol from the release devices.

Year	New lure	Installed on the beginning of season			Installed in the middle of season		
	ø weight±SE (g)	ø weight±SE (g)	evapored amount (g)	evapored daily (g)	ø weight±SE (g)	evapored amount (g)	evapored daily (g)
2011		from 24.3.2011 (**169 days**)			from 17.6.2011 (**85 days**)		
	123.82±1.70	76.09±4.36	**47.73**	0.282	96.51±2.80	**27.31**	0.321
2012		from 23.3.2012 (**168 days**)			from 15.6.2012 (**84 days**)		
	119.41±0.60	57.27±5.49	**62.14**	0.370	76.05±2.22	**43.36**	0.516

Explanations: ø – mean, SE – standard error.

curred in early April and the second peak occurred in early summer (Fig. 2H). Overall activity of *T. signatum* in 2010 and 2011 was low and very few were captured (Table 2). The number of *T. signatum* increased in 2012 and the peak occurred in early April (Fig. 2I). *P. cylindrus* was not recorded in 2010. In 2011 and 2012, a few specimens were captured but only in July and August (Fig. 2A).

Comparison of UHR ethanol trap catches versus control
In total, traps baited with UHR ethanol lures captured significantly higher numbers of ambrosia beetles compared to unbaited control traps (Fig. 3). The average number of beetles caught in 2010 in ethanol-baited traps was 21.63 specimens/trap as compared to only 4.21 specimens/trap in the control ($P < 0.0001$, Tukey HSD test). This difference was also confirmed in 2011 (UHR ethanol 21.59 specimens/trap, control 4.42 specimens/trap) and in 2012 (UHR ethanol 15.45 specimens/trap, control 3.29 specimens/trap) (ANOVA, $P < 0.0001$, Tukey HSD test) (Fig. 3). There was no significant difference observed between the number of beetles captured in traps baited with UHR ethanol and other chemicals vs. UHR ethanol by itself (Tukey HSD test, all NS).

Elution of ethanol from the release devices
The average weight of UHR ethanol lures at the beginning of the season was 120 g (mean weight 123.82 ± 1.70 g in 2011 and 119.41 ± 0.60 g in 2012) (Table 3). Release rates of the lures installed at the beginning of the 2011 season (47.73 g eluted from the release device over 169 days in the field) was 0.282 g/day, whereas those installed in the middle of the 2011 season released 0.321 g of ethanol per day (27.31 g eluted over 85 days in the field). Release rates of lures placed at the beginning of the 2012 season (62.14 g eluted over 168 days) was 0.370 g/day while lures installed in the middle of the season showed a total weight loss of 43.36 g (0.516 g/day) after 84 days (Table 3).

Discussion

This experiment demonstrates the utility of using UHR ethanol lures for the first time to trap ambrosia beetles in Slovakian oak forests. These lures proved to be effective in attracting almost all ambrosia beetle species (Pfeffer 1989) in the oak study area. This experiment also supports the results of other ambrosia beetle monitoring trials which have been conducted only a few times in Europe (Markalas & Kalapanida 1997; Bouget & Noblecourt 2005).

Several studies suggest that ethanol is produced from fermentative processes when trees respond to a number of environmental stresses including flooding, drought, or high levels of pollutant gases (Kimmerer & Kozlowski 1982; Naik et al. 2010). Low oxygen conditions arising in stressed trees can also lead to ethanol production and subsequent attack by bark and ambrosia beetles (Miller & Rabaglia 2009). Kelsey & Joseph (2001) reported that all water-stressed branches of Douglas-fir trees contained significantly higher ethanol concentrations. As ethanol is only produced in sufficiently moist sapwood and phloem tissue, it apparently signals suitable host material for ambrosia beetles and their associated fungi (Klimetzek et al. 1986). Montgomery & Wargo (1983) confirmed that ethanol was produced by trees under stress and consequently was used as a host location signal by beetles. They claim that ethanol production in response to stress would be fairly rapid and it is possible that ethanol may also be invoked by beetle infestation. Coutts & Armstrong (1976) also showed that waterlogging of tree roots could lead to substantial increase in root ethanol levels. Crawford & Baines (1977) found that stem ethanol levels were strongly correlated with ethanol levels in roots. Apparently, ethanol perception represents a crucial mechanism in host allocation for many living insects, particularly ambrosia beetles which depend on the cultivation of fungal symbionts (Klimetzek et al. 1986).

In 2011 we also tested other three chemicals in combination with UHR ethanol lure (Table 1). Some studies demonstrated that E-(2)-hexanal is a common compound within the bark of *Q. robur* (Vrkočová et al. 2000) and conophthorin as another non-host volatiles which is present in the bark of many hardwood trees (Zhang et al. 2002). Also 2-methyl-3-buten-2-ol was found in the bark of deciduous trees (Zhang et al. 2012). Ethanol is a common attractant to many wood boring insects. This was the reason for testing these three additional lure combinations. The purpose was to increase catches of ambrosia beetles but as results suggests without any additional effect.

In this experiment *X. saxesenii* was the most commonly captured insect in selected Slovakian oak forests. As demonstrated by other experiments in Europe and the USA, *X. saxesenii* is attracted to ethanol (Roling & Kearby 1975; Montgomery & Wargo 1983; Klimetzek et al. 1986; Markalas & Kalapanida 1997; Flint et al. 2007). Similar to other studies in European oak forests (Markalas & Kalapanida 1997; Bouget & Noblecourt 2005; Lakatos & Kajimura 2007), *X. saxesenii* and *A. dispar* were the most frequently captured species during our study period.

Prior to initiating an insecticide control program, ethanol baited traps are used in the USA to monitor the flight activity of *X. saxesenii* and *X. germanus* in horticultural tree nurseries (Oliver & Mannion 2001; Bambara et al. 2008; Miller & Rabaglia 2009). Ethanol baited funnel traps are utilized in the USA and Canada to monitor various bark and ambrosia beetles (Coyle et al. 2005; Humble et al. 2010; Noseworthy et al. 2012; Bullas-Appleton et al. 2014). Conversely, ethanol baited traps are not used to detect or monitor ambrosia beetles in Slovakia or many other European countries.

Bouget & Noblecourt (2005) indicated that the dominance of *X. germanus* in some regions highlighted the current expansion of this exotic non-native species in Western Europe. This ambrosia beetle was discovered for the first time in Slovakia during this experiment (Galko 2013) and the number of beetles captured is increasing every year (Table 2). In 2013, this trend continues as 322 *X. germanus* specimens were captured in a similar experiment. In 2014, for the first time in Slovakia, we found heavily infested logs of beech and oaks by *X. germanus* (Galko, unpublished data). *X. germanus* is native to eastern Asia and is among the most economically important exotic ambrosia beetles in USA nurseries (Oliver & Mannion 2001; Ranger et al. 2010). Miller & Rabaglia (2009) claimed that in some experimental plots more exotic non-native species were caught compared to the number of native ambrosia beetles. Coyle et al. (2005) and Oliver & Mannion (2001) found in South Carolina and Tennessee that the percentage of non-native beetles in ethanol baited traps was 88% and 74%, respectively. The impacts of *X. germanus* in central Europe may be different than in the USA. Although the introduction of *X. germanus* into Europe probably occurred some time ago, the damage caused by this beetle is negligible and it is currently considered a secondary pest species in Europe (Lakatos & Kajimura 2007). Because there was an increase in trap catches of *X. germanus* each year, the population dynamics and impacts of this insect should be monitored over time to determine if this insect will remain as a secondary pest in Slovakia or if it will become a primary pest as it has become in some regions of the USA.

We evaluated data of seasonal flight patterns of nine of the most common ambrosia beetles in the study area (Fig. 2). The most commonly captured beetle was *X. saxesenii.* The peak of its activity was similar in each year of the study period. The captures occurred from early May until late September. The recorded peak activity corresponded with the Markalas & Kalapanida (1997) study from Greece, but the beetle flight activity started earlier in March and lasted until late October. The longer flight period in Greece is probably the result of the warmer Mediterranean climate (Köppen climate classification (KCC) *Csa*). In South Carolina, the humid subtropical climate (KCC *Cfa*) resulted in an even longer flight period for *X. saxesenii,* which occurred from late February until December (Coyle et al. 2005).

The peak activity of *A. dispar* in our study occurred in late April and continued until July/early August. This species is univoltine (Schwenke 1974) and its flight is initiated when the maximum daily temperatures reach 18–20 ℃ (Mani et al. 1992). Markalas & Kalapanida (1997) reported that in Greece, this pest occured from early April until July, with peak activity in May.

Schwenke (1974) stated that *X. monographus* produced two generations per year (the first in March–April, the second in June–July). However, our findings (Fig. 2F) and also those from Greece (Markalas & Kalapanida 1997) do not confirm these conclusions.

Only a few *X. germanus* specimens were captured between May and August. In the USA, the peak activity of this pest varies from region to region. For example, its activity culminates in May in Ohio, late March–April in Tennessee and April–May in Virginia (Reding et al. 2010).

We found that the average amount of ethanol eluting from the lure was 0.282 g/d in 2011 and 0.370 g/d in 2012. Data from the producer of this lure state an average release rate of 260 mg/day at 20 ℃. Klimetzek et al. (1986) tested ethanol (near Freiburg, Germany) and found release rates from different dispensers ranging from 0.024 g/d to 6 g/d. The differences in release rates can be caused by several factors such as type of dispenser, amount of active compound, temperature, trap shape, weather, etc. Miller & Rabaglia (2009) in the south-eastern USA observed an average release rate of 0.6 g/d at 25–28 ℃. Some authors reported that high release rates may have an inhibitory effect and reduce the number of captured ambrosia beetles (Salom & McLean 1990; Ranger et al. 2011). The attractiveness of acetone and acetaldehyde were also been tested in these studies, but they were not attractive to any ambrosia beetles. Both of these compounds can be formed in plants by oxidative metabolism of ethanol (Cossins 1978). Ranger et al. (2010) tested low release ethanol lure (30 mg/d at 20 ℃) and found that ethanol is the most attractive stress-related volatile to *X. germanus.* Methanol baited traps were slightly attractive to *X. germanus*, whereas traps baited with acetaldehyde and acetone were not attractive to any ambrosia beetle.

In conclusion, the present study demonstrates the effectiveness of UHR ethanol lures in attracting ambrosia beetles in an oak forest in W Slovakia. Further research is required to determine if similar patterns will occur in other parts of Slovakia or Europe. Our research was conducted in hilly conditions within a closed forest ecosystem, away from major roads, railways and urban centres. Therefore we assume that the introduction of *X. germanus* occurred a few years before our discovery. As Grégoire et al. (2001) suggested, we should continue to monitor the dispersal and population dynamics of *X. germanus* in the years that follow in order to ascertain its impact to host trees.

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